



Black cumin Potential Anticancer effect of Black Cumin seed (*Nigella sativa* L.) extracts as determined by Cytotoxicity test against Larvae of *Artemia salina* Leach using Brine Shrimp Lethality Test (BSLT)

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Abstract : Black cumin plant seed or black seed (*Nigella sativa* L.) is among the most commonly used spices. This plant is grown mostly for its spicy seeds. Studies have reported that black cumin seeds have high levels of antioxidant that correlates with anticancer activities. The current study aims to determine the cytotoxic effect of black seed extracts on brine shrimp larvae (*Artemia salina* Leach) using Brine Shrimp Lethality Test (BSLT). Black cumin seed extract was obtained through percolation method using an extraction solvent (ethanol 96%). The cytotoxicity test was performed at 0 ppm concentration (control) and 10, 50, 100, 250 and 500 ppm concentrations of black seed extracts. We use 10 Brine Shrimp larvae per tube and repeated the test three times for each test group. Our results show that ethanolic extract (90%) of black cumin seeds (*Nigella sativa* L.) has a strong cytotoxic effect on *Artemia salina* larvae with the LC₅₀ value of 107.2 ppm.

Keywords : Cytotoxic, black cumin seed (*Nigella sativa* L.), *Artemia salina* Leach BSLT.

Introduction

Cancer is a leading cause of death worldwide, especially in North America, Australia, and Europe. As for Indonesia, cancer ranked third in terms of incidence. The most common treatment of cancer is chemotherapy. The problem experienced by cancer patients undergoing chemotherapy is that chemotherapy drugs kill not only cancerous cells but also the normal ones. This, in turn, causes other treatment-related problems, such as decreased organ function, hair loss, reduced hemoglobin, nausea and vomiting.¹

These problems make patients anxious and afraid to undergo the therapy. Therefore, some of them choose alternative treatments instead. In addition, the high cost of cancer treatments and the fact that there are cases of cancers that can be cured with medicinal plants lead to growing shift toward alternative treatments with herbal medicine.²

One of the medicinal plants with the potential to inhibit the growth of cancer cells is *Nigella sativa*. Known also as black cumin, *Nigella sativa* is an annual flowering plant in the family Ranunculaceae.³ Black cumin seeds have long been used, especially in the Middle East and Southeast Asia, for its therapeutic benefits. Traditionally, black cumin seeds are used as diuretic, diaphoretic, anthelmintic, and carminative drugs.⁴ However, it has been reported in a number of studies that black cumin seeds have also anti-bacterial, anti-parasitic, anti-inflammatory, immunomodulatory, antihypertensive, anti-tumor, antioxidant and anti-cancer properties.^{5,6} Phytochemical tests show that black cumin seeds contain essential oils, fatty acids, alkaloids, flavonoids, tannins, saponins and terpenoids.^{7,8,9}

Mariod et al. (2009) reported that black cumin seed has a strong antioxidant activity after conducting DPPH and Beta Carotene-Linoleic Acid tests to determine the antioxidant activities of black seed extract.¹⁰ Other similar studies also stated that black seeds extract concentrations of 1%, 2%, 3% 4% and 5% have strong antioxidant activities.¹¹ The antioxidant activity of a test compound is predicted to correlate with anticancer (cytotoxic) activity. This accords with a theory stating that antioxidant activities of a compound may inhibit free radical chain reaction, an absorption of Reactive Oxygen Species that reduces the risk of cancer.¹²

The potential anticancer activities of black seeds remain largely undetermined. The available collection of literatures has not explain how to process black cumin seeds to generate optimum anticancer activity.¹³ Therefore, it is necessary to conduct preliminary research to determine the potential anticancer activity of black seed extracts by conducting cytotoxicity assay with *Artemia salina* larvae using BSLT (Brine Shrimp Lethality Test).

Experiment

Materials

Black cumin seeds (*Nigella sativa* L.), ethanol 96%, aqua dest, seawater, aluminum foil, dimethylsulfoxide 1% (DMSO 1%).

Test Object

Artemia salina larvae are used as our test objects. They were obtained from the Regional Centre for Brackishwater Aquaculture Development (BBAP Ujung Batee, Aceh Besar) in the form of eggs for subsequent hatching.

Preparation of Extracts

The extract is prepared through percolation procedure to filter 400 g of black seed powder that has been macerated for 3 hours. The mass is then transferred into percolator; ethanol 96% is added in amount sufficient to soak the simplicia and the percolating fluid remains above the mass; the lid of the percolator is closed and then let it remains closed for 24 hours. Open the percolator lid and let the extract fluid drops at 20 drops per minute and add ethanol 96% repeatedly. Percolation is halted if the out flowing filtrates become colorless. The percolate is then evaporated by means of vacuum rotary evaporator to obtain a thick extract.¹⁴

The Hatching of *Artemia salina* Eggs

For *Artemia salina* Leach, a vessel with two spaces is prepared. A lamp is placed to generate warm temperature for hatching in one space, a sufficient amount of sea water is poured into the other. 50-100 mg shrimp eggs were poured into sea water for hatching. The space that contains shrimp eggs is covered with aluminum foil, and the lamp is turn on for 48 hours for hatching. The 24 hours hatched larvae will tend to swim toward the light source. The shrimp larvae to be tested are taken up using pipette.¹⁵

Procedure of Cytotoxicity Test

Cytotoxicity test is conducted using BSLT method. The extract (test solution) is divided into 6 groups of concentration: 500 ppm, 250 ppm, 100 ppm, 50 ppm, 10 ppm, and 0 ppm. Then put the 48-hours old *Artemia salina* larvae into the respective concentrations, each with 10 larvae. Do this three times (Tripto). The reaction tubes are placed in an open area for 24 hours. Count the surviving larvae in each reaction tube. The standardized

criterion for ensuring the death of larvae is no movement during observation. Count the percentage of dead larvae and do a probit analysis to calculate the LC50.¹⁶

Results and Discussion

Cytotoxic activity test using the BSLT (Brine Shrimp Lethality Test) method was performed as a preliminary study of anticancer. The observation parameter is the death of *Artemia salina* larvae. Larvae are said to die if they do not respond to a touch. The results show that the death of *Artemia salina* increases with higher concentration of extracts (Table 1). The highest percentage of larval mortality was found in the extract concentration of 500 ppm, and the lowest percentage of larval mortality was found in the extract concentration of 10 ppm.

Tabel 1. Observation Result of Cytotoxic Test of Black Cumin Seed Extract (*Nigella sativa* L.) on *Artemia salina* Leach larvae.

Treatment	Concentration (ppm)	Average Larval Mortality	Percentage of Larval Mortality (%)
Control (P0)	0	0	0
Black Cumin Seed Extract (P1)	10	1	10
Black Cumin Seed Extract (P2)	50	3	30
Black Cumin Seed Extract (P3)	100	5	50
Black Cumin Seed Extract (P4)	250	7	70
Black Cumin Seed Extract (P5)	500	8	80

Differences in the percentage of larval mortality in table 1 might be caused by differences in the level of chemical compound of the extracts; the higher the extract concentration, the stronger the cytotoxic effect in the test solution would be. Several studies reported that black cumin seeds contain essential oils, fatty acids, alkaloids, flavonoids, tannins, saponins and terpenoids.^{7,8,9} No larval mortality was found in the control group (P0). This is because no extracts were added to the test solution of the control group. For this reason, it is safe to say that larval dead is caused solely by the addition of black cumin seed (*Nigella sativa* L.) extracts. Probit analysis (Table 2) is necessary to compute the LC50 of black cumin seed extracts administered to *Artemia salina* larvae.

From the data presented in Table 2, linear equation is obtained: $Y=1.28x+2.40$, with LC50 value of 107.2 ppm. The obtained LC50 value indicates that the ethanolic extract of black seed (*Nigella sativa* L) is toxic and has potential anticancer properties because its LC50 value < 1000 ppm.¹⁷

Tabel 2. Data hasil perhitungan probit efek toksik ekstrak Biji Jintan Hitam (*Nigella sativa* L.)

Concentration (ppm)	log 10 (Concentration)	% Mortality	Probit
10	1	10	3.72
50	1.699	30	4.48
100	2	50	5
250	2.398	70	5.52
500	2.699	80	5.84

The cytotoxicity of black cumin extract is thought to come from its content of Tymoquinone. Thymoquinone is a phytochemical compound belonging to class of terpenoids (monoterpene) found mostly in black cumin seeds by $\pm 7,8-13,7\%$.¹⁸ Thymoquinone has been shown to have potential anti-cancer remedy, as has been proven in a number of studies.¹⁹ El-Najjar in Randhawa et al (2011) reported that Tymoquinone has been proven to inhibit the proliferation of human cancer cells (Caco-2, HCT-116, LoVo, DLD-1 dan HT-29).²⁰ A

study by Woo et al (2011) also provides evidence that Tymoquinone of black cumin seeds may inhibit the growth of breast cancer cells MCF-7, MDA-MB-231 dan BT-474.²¹ Tymoquinone is known to have an antiproliferation effect by reducing cell oxidative stress and, thus, triggering cell apoptosis.^{22, 13}

From this study it can be concluded that black cumin seeds exhibit toxicity against the larvae of *Artemia salina* Leach. However, further studies are necessary, especially those using cancer cell culture as the object of study. This preliminary study is expected to open up opportunities for black cumin seed extract to be used as a subject of anticancer research, considering that the pattern of larval development of *Artemia salina* is similar to that of cancer cells, which is fast and uncontrolled.

Conclusion

This study proves that black cumin seeds have toxicity against larvae of *Artemia salina* Leach with an LC₅₀ value of 107.2 ppm.

Recommendation

We recommended future researches to focus on human cancer cell culture, both in vitro and in vivo, in order to determine the anticancer effect of black cumin seeds (*Nigella sativa* L).

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